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Ecotoxicological responses at the population level

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Abstract

Ecotoxicology aims to recognize, analyse and predict effects of potentially toxic chemicals at higher levels of biological organization, such as the population level. This paper reviews various theoretical approaches that have been undertaken to relate properties of individual organisms (age, body size, fertility) to properties of populations (age-distribution, growth rate, biomass turnover). Population analysis may then be used to answer the question whether populations may respond more sensitively to toxicants than individual organisms. An example of the theory is given using data obtained from life-cycle toxicity experiments on springtails (Collembola) and mites (Oribatida). Two species representing different life-styles accumulate cadmium from food in different ways: the mite Platynothrus peltifer *excretes Cd very slowly while the springtail* Orchesella cincta *is able to excrete cadmium rapidly. The species with the higher equilibrium body burden* (P. peltifer), *however, is more resistant in terms of the* LC_{50}, *although it is more sensitive in terms of effects on reproduction, compared to* O. cincta. *Population responses are determined mainly by effects on reproduction. It is argued that the classification of life-forms of Collembola, which joins morphology with life-history strategies, forms a good basis for classifying ecotoxicological responses of soil invertebrate populations.*

1. Introduction

The aim in environmental toxicology is to recognize, analyse and predict possible negative effects of man's activities on the environment, especially in relation to the release of toxic substances and in relation to the increase of availability of mineral nutrients in one place or their decrease in another place. This chemical alteration of the environment is associated with various human activities such as crop production, industry, housing, traffic and the like. Above all, these activities have important socio-economic dimensions. It has been a great achievement of the World Commision on Environment and Development to analyse the link between economics and environment using the concept of Sustainable Development (WCED 1987). In this book, we discuss a much more narrow issue, but we

are all aware of the fact that our relatively small-scaled research has important socio-economic implications.

In this contribution, I would like to analyse one of the very small steps that has to be taken to fulfill the aims of environmental toxicology: the relationship between toxic effects on individuals of a certain species, and the response of a population, inhabiting a certain area, of the same species. This small step is essential to understand any further steps in the extrapolation to ecosystem disturbances by toxic chemicals.

At the level of populations, we have to deal with several important characteristics that cannot be measured on individuals. In population ecology, several approaches have been laid out to find the relationships between individual characteristics and population characteristics (Kooijman 1985). In doing so, population ecology has developed into a rigorous discipline, often using mathematical models to predict population responses. In my opinion, this mathematical, quantitative approach is also needed in ecotoxicology.

2. Population performance indices

Population characteristics are called 'emergent' when they are typical for the population level and cannot be seen as properties of lower levels. Table 1 lists several of these emergent properties of populations. Three of them: the intrinsic rate of increase, the intrinsic rate of biomass turnover, and the carrying capacity, are also used in ecotoxicology. This paper will review their theoretical basis and then discuss some applications.

Table 1 *Emergent properties of populations*

Density	Number of individuals per unit habitat
Sex ratio	Ratio of males to females
Age-structure	Frequencies of juveniles and adults
Intrinsic rate of increase	Capacity to grow under non-limiting conditions
Carrying capacity	Maximum density achieved under limiting conditions
Biomass	Amount of living organic matter per unit habitat
Production	Amount of biomass added to the population through body-growth and reproduction, per time unit
Biomass turnover	Ratio of production to biomass
Genetic variation	Variability due to inter-individual differences in the genome

The use of the intrinsic rate of increase as a measure of population performance under toxic stress is motivated by the fact that, according to Fisher's fundamental theorem of natural selection, it is a measure of Darwinian fitness for age-structured populations. Euler's equation gives the relationship between survival as a

function of age (l_x), fertility as a function of age (m_x), and the intrinsic rate of increase (r):

(1)
$$\sum_{x=0}^{\infty} l_x m_x e^{-rx} = 1$$

The usefulness of this expression in ecotoxicology has been demonstrated by various authors (Day & Kaushik 1987, Meyer *et al.* 1987, Seitz & Ratte 1991). The equation may be used to integrate the effects of a chemical on survival and mortality, in an ecologically relevant way, into a single measure. It is ecologically relevant because it takes account of the life-cycle peculiarities of the species under study. There are however two drawbacks to using only the intrinsic rate of increase r and I will demonstrate these using two of the other measures pointed out in Table 1.

Using the same life-table approach, it is possible to estimate the intrinsic biomass turnover of a population. When an age-structured population has achieved a stable age distribution, the total production of the population, that is the amount of biomass produced per time unit through growth and reproduction, will increase at the same rate as the total population abundance. Consequently, the ratio of production to biomass (abbreviated to P/B) will be constant. This ratio is called the intrinsic biomass turnover rate. It is possible to predict the biomass turnover rate, if the survival function, the body-growth function, and the fertility schedule are known, using equation (2):

(2)
$$\frac{P}{B} = \frac{\sum_{x=0}^{\infty} (l_x - l_{x+1}) w_x e^{-rx}}{\sum_{x=0}^{\infty} l_x w_x e^{-rx}} + r$$

where:
l_x = survival from birth to age x
w_x = body-weight at age x,
and r is calculated using equation (1).

Biomass turover depends on three variables: l_x, m_x and w_x, instead of two, because it also takes account of the weight of the animals as a function of age. The derivation of the expression is given in Van Straalen & De Goede (1987). It may be seen from equation (2) that the biomass turnover rate is always greater than r, because it equals a positive quantity plus r. This is easy to understand, because even if r is zero, the population does not grow at all and the biomass is constant, there will always be some production: organisms will take up food, grow, reproduce and eventually die. Even a stationary population has a certain production of biomass, which is removed by death at the same rate as it is produced by growth and reproduction.

The use of the biomass turnover rate is not so common in ecotoxicology, partly because the theory is not in the textbooks, but has been developed by Al-

denberg (1986) and Van Straalen & De Goede (1987). It may be relevant though, because in toxicity tests there is often a negative relationship between growth and reproduction. For example, Van Gestel *et al.* (1992) have shown that, using toxicity tests with earthworms, in experiments where the worms produced cocoons at a high rate, they did not grow so well, while in other experiments, when reproduction was not so high, the worms increased in body size considerably. If a chemical inhibits reproduction, growth might be stimulated, and vice-versa. Equation (2) shows how all these factors might be considered in an ecologically relevant way. Biomass production, especially for earthworms, is very relevant, because these animals are eaten by many surface-active birds, such as thrushes.

The third population measure to be discussed is carrying capacity, often denoted by K. Equation (3) represents the logistic equation for population growth:

$$N(t) = \frac{K}{1 + \left[\frac{K - N_0}{N_0}\right] e^{-r_m t}} \qquad (3)$$

where:

N(t) = population size at time t,
N_0 = initial population size,
K = carrying capacity,
r_m = maximal population growth rate.

Of course no one population will grow exponentially forever. After some time the food supply will be limiting, growth will be stopped and the population size will be more or less in equilibrium with the environment. The carrying capacity, which is then achieved, will measure the ability of a population to maintain itself in a situation where there is shortage of food, intense competition, and selection pressure for efficiency, rather than for high production.

The intrinsic rate of increase is actually relevant only at low densities, when there is plenty of food, and there are no restrictions to high productivity. In equation (3) the rate of increase depends on density; its maximum value r_m is achieved when N approaches zero.

The theory of the logistic equation is not so well developed, because it is not so clear what exactly is the relationship between the individual measures mortality, fertility and body-growth, and the carrying capacity K. Nevertheless it has been applied in several examples in ecotoxicology, mainly in aquatic studies (Halbach *et al.* 1983, Van Leeuwen *et al.* 1987).

One of the questions which has been posed by ecotoxicologists is whether it is possible that populations respond more sensitively to toxicants than individual organisms? The issue was raised in an article by Halbach *et al.* (1983), who maintained that population measures may act as a magnifying glass; some very subtle effects cannot be observed in individual organisms, but on the level of the population they will appear because this is composed of many individuals. In this way toxic levels may be exaggerated by populations. If this is true then it will seriously

invalidate any predictions made on the basis of single species tests, looking only at mortality and reproduction, because in this way we cannot predict effects on the level of populations from simple laboratory experiments.

3. Soil invertebrates

The soil community is very diverse and it holds many species. Even more importantly, it covers species with a great variety of life-cycles. Some of them have a very rapid turnover, high fertility and short life-time, while others may live for more than a year with a low turnover. The soil community provides us with an excellent opportunity to test the theory on the influence of life-tables and the importance of population level effects, by comparing the effects in different species. For the purpose of this contribution, I will present the results for only two species which I chose more or less at random out of the community of invertebrates: a springtail, *Orchesella cincta*, and an oribatid mite, *Platynothrus peltifer*.

Springtails or Collembola are primitive insects which are abundant in forest soils, especially when there is a good cover of litter. The density of the total springtail fauna may vary between 5,000 and 50,000 per square meter in forest soils of the temperate zone, but their density seems to be lower in tropical ecosystems. Usually there are some 20 to 30 species within a single research plot, and several thousands of species have been described for Europe and America. Oribatid mites are also a very numerous group of soil arthropods. Total Oribatida may amount to 100,000 animals per square meter, while there are several thousands of species.

The great variety of the arthropods in organic soils has been a drawback for their study, because many of them are not so easy to identify. The taxonomical problems in the tropics are still considerable and it takes a specialist to make reliable identifications.

Table 2 *Contrasting life-histories of two forest floor arthropod species*

	Orchesella cincta	*Platynothrus peltifer*
Average life-time (months)	0.5 - 1.0	7.8
Survival to reproduction	0.02 - 0.04	0.3
Fertility (eggs/female/month)	33 - 46	1 - 3

The two species chosen represent two different life-styles, which actually form a continuum ranging over all the species. Table 2 presents an overview of the life-histories of the two contrasting species. Under natural conditions, *Orchesella cincta* has an average life-time of only a few weeks, up to one month, while *Platynothrus peltifer* may live on average for 7 to 8 months, and some of them may live for more than a year. It is extraordinary that a small animal like *Platynothrus peltifer* which is only 1 mm long, lives for such a long time. Usually one would

expect that the smaller a species is, the shorter it will live. This trend is certainly not true for soil microarthropods.

There is also a great difference with respect to the survival probability to reproduction. Only 2 to 4 percent of *Orchesella cincta* hatchlings will reach the adult stage, while for *Platynothrus*, this is 30%. With respect to fertility, expressed as the number of eggs per female per month, the opposite is true: *Orchesella* females may produce as many as 48 eggs, while *Platynothrus* lays only 2 eggs at a time during one month.

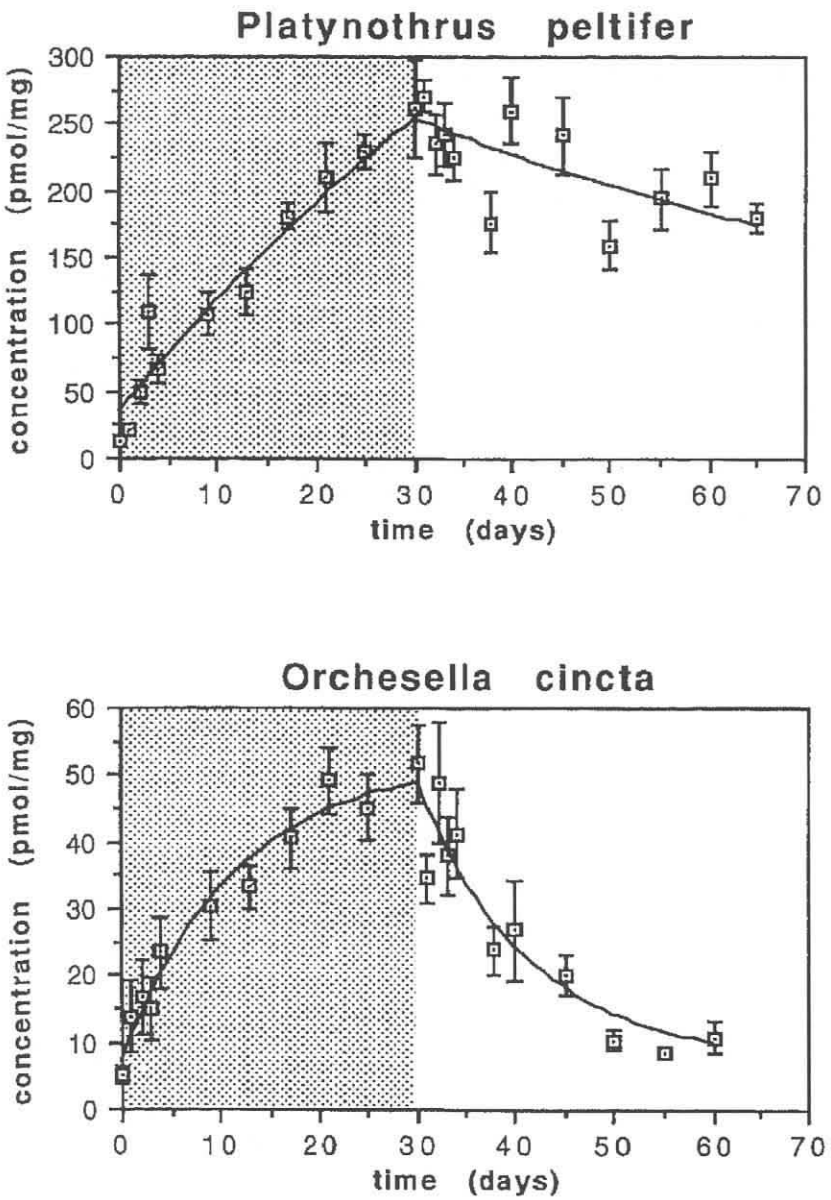

Figure 1 *Accumulation and elimination of cadmium by two species of soil arthropods, when exposed to cadmium contaminated food (± 20 µg/g) during the shaded period, followed by clean food*
Adapted from Janssen *et al.* (1991)

These species thus really represent two different life-styles, varying from very fertile, short-lived species to longer living species producing not so many eggs. These different life-styles are correlated with various physiological characters, one of the most important being the metabolic rate. Using the Cartesian diver respirometer, it is possible to measure the metabolic rate of these animals, expressed as mL of oxygen consumed per gram of body-weight per hour. In a study conducted by Janssen & Bergema (1991), the metabolic rate was 542 mL/g/h for *O. cincta* and 405 mL/g/h for *P. peltifer*, both at 20°C.

These two species were used in chronic toxicity tests using cadmium as the toxic compound. Cadmium, belonging to the heavy metals, is emitted into the environment by various human activities, most notably the smelting of zinc ore. All over the world there are foundaries which emit this toxic element into the air.

When heavy metals are deposited from air pollution onto the ground they tend to be bound by the upper organic layers of the soil. This is because dead organic matter has a great capacity to bind divalent ions, due to various functional groups in humus molecules. Unfortunately, saprotrophic invertebrates have to eat this material and by doing so, they are exposed to high doses of metals.

4. Kinetics of cadmium

Figure 1 shows the results of experiments conducted by Janssen *et al.* (1991), designed to quantitatively estimate the uptake and elimination of cadmium by the two selected invertebrates. During the shaded period, the animals were exposed to cadmium-contaminated food for 30 days. In the rest of experiment, the animals were given clean food, for another 30 days. Obviously, the cadmium concentration increases when the food is contaminated, while it decreases again when the food is clean. The solid line represents a curve fit based on a one compartment model.

Table 3 *Summary of cadmium kinetic parameters, estimated from uptake-elimination experiments (Figure 1), using a one-compartment model*

Parameter	*Orchesella cincta*	*Platynothrus peltifer*
Cadmium assimilation from ingested food (%)	9.4	17.2
Cadmium excretion rate relative to body burden (d^{-1})	0.087	0.013
Equilibrium concentration relative to food (BMF)	0.22	3.4

After Janssen *et al.* (1991).

From these experiments we may estimate some parameters characterizing the uptake and elimination of cadmium in these two species. These are summarized

in Table 3. *Orchesella* will assimilate 9.4% of the cadmium consumed, while the corresponding figure for *Platynothrus* is 17.2%. Both figures are significantly higher in comparison to humans, who will assimilate 4.8% of the cadmium consumed with the food (Nordberg & Kjellstrom 1979).

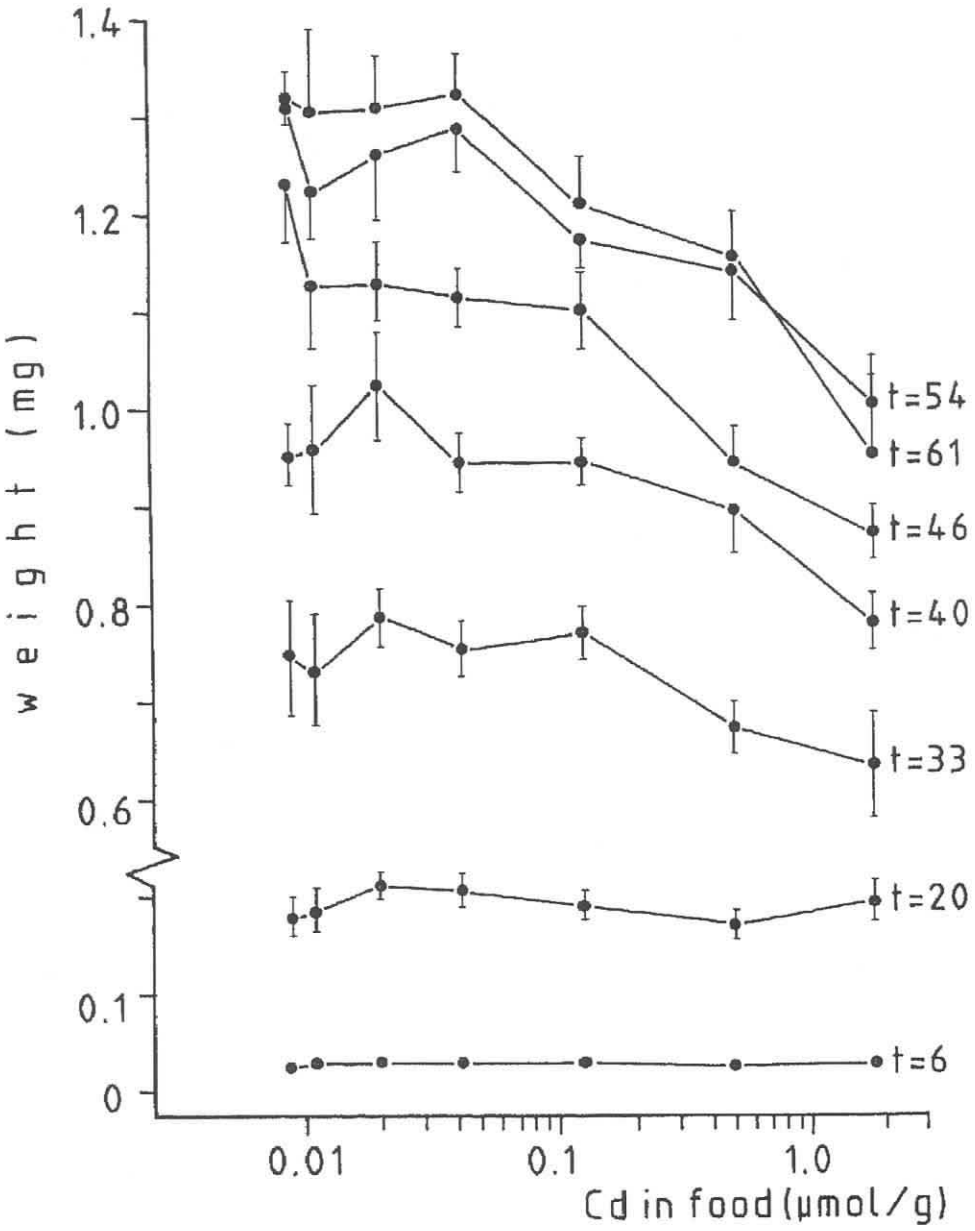

Figure 2 *Growth of female springtails,* Orchesella cincta, *exposed to various levels of cadmium contaminated food for a period of 61 days. Means are given with their standard errors*
Adapted from Van Straalen *et al.* (1989)

Excretion is 0.087 d^{-1} for *Orchesella* and 0.013 d^{-1} for *Platynothrus*, so the second species not only assimilates cadmium more efficiently, it also excretes the material less efficiently. This will cause a higher body burden: the equilibrium concentra-

tion is 3.4 times the concentration in the food for *P. peltifer*, while *O. cincta* keeps its body concentration almost 5 times below the food concentration (Table 3).

Summarizing, there is a clear difference between the species in kinetics, which of course relates to a difference in physiology. Care should be taken, however, in not to base the conclusions only on kinetics. A species having a higher body burden is not necessarily more sensitive to the metal, it might even be more resistant. Next to the kinetic experiments we thus also have to consider toxicity experiments to analyse the relationship between external concentration, internal concentration and toxicity.

5. Toxicity of cadmium

Figure 2 gives a series of dose-effect curves for growth of *Orchesella cincta*, obtained for various levels of Cd in the food, after increasing exposure times. The curves are all on top of each other because the animals increased in weight during the experiment. It may be seen from the graph that after 33 days there is an effect of cadmium on the growth of those animals at the two highest cadmium concentrations, and further on in the experiment, the effect also becomes visible at lower concentrations.

From a graph like this, one may estimate the lowest concentration causing a negative effect on growth, as well as the highest concentration causing no effect. The latter is called the NOEC, no observed effect concentration. Using appropriate statistical methods (Williams 1971) the NOEC is estimated as 4.7 μg/g.

In a similar way, one may analyse the effects of cadmium on reproduction. It appears that cadmium does not have very drastic effects on the reproduction of *Orchesella*, it has a slight effect because of the decreased body-size. This is because smallller animals lay smaller clutches; the primary effect, however, is on growth.

Figure 3 gives a response curve for fertility of *Platynothrus peltifer*, expressed as the number of eggs per female over 9 weeks. This species shows a rather strong effect of cadmium: at the highest concentration, no eggs at all were laid during the whole experiment, however, at the 3rd concentration, there is a significant decrease of egg production. The NOEC is estimated as 2.9 μg/g. The toxicity of Cd in *P. peltifer* may be be related to a disturbance of the Zn metabolism. This species has a very high Zn concentration and under Cd exposure Zn is lost from the body (Van Straalen *et al.* 1989).

The data obtained from these life-cycle toxicity experiments can now be used for calculating population parameters. As a good ecological background knowledge exists for these species, it is possible to include mortality data from field studies to arrive at realistic estimates for the population growth rate.

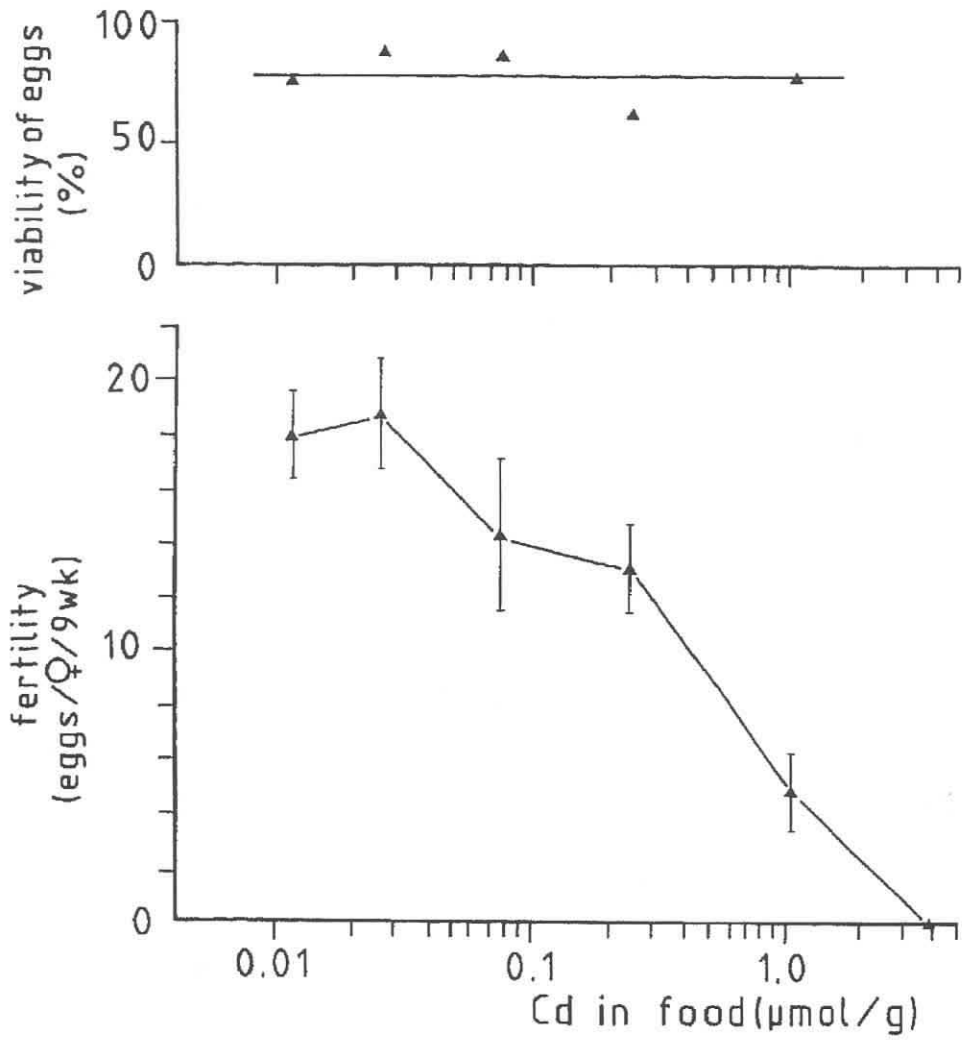

Figure 3 *Reproduction of oribatid mites,* Platynothrus peltifer, *exposed to various levels of cadmium contaminated food for a period of 9 weeks. Means are given with their standard errors*
After Van Straalen *et al.* (1989)

6. Population responses

Figure 4 provides a dose-response curve for the population growth rate r. When these is little Cd in the food the population growth rate is close to zero, because in the field these animals will have more or less stable populations from year to year. When we add cadmium, the population growth of *Orchesella* remains positive up to a rather high concentration. However, the population growth of *Platynothrus* is already negative at the 3rd concentration level.

These responses may be relevant when extrapolating to the field situation. At contaminated sites, *Platynothrus* will be in danger because it is not able to maintain its population due to a negative population growth, while *Orchesella* may still be present.

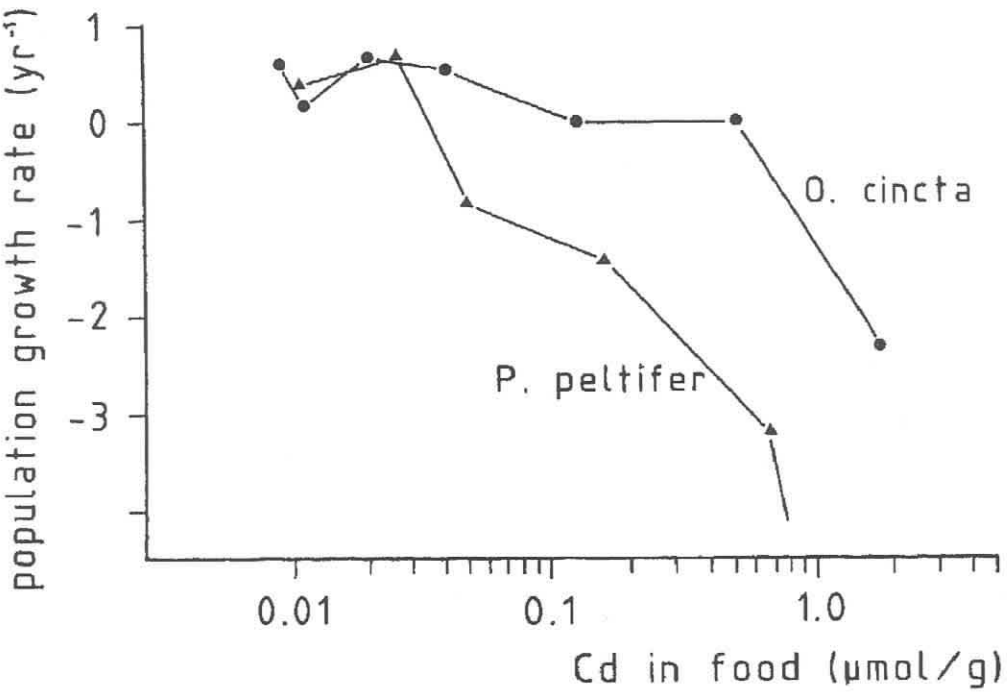

Figure 4 *Population growth rate of* O. cincta *and* P. peltifer, *calculated from toxicity data combined with life-history data from the field, as a function of cadmium in the food*
Source: Van Straalen *et al.* (1989).

Table 4 summarizes the results of the toxicity experiments. When comparing the two species, it appears that *Platynothrus* has the highest LC_{50}. Despite the fact that it accumulates Cd to a higher degree (Table 3), it is actually more resistant, in the sense that it takes a rather high concentration in the food to induce mortality.

Considering the NOEC for the sublethal parameters growth and reproduction, these are much lower than the LC_{50} and they do not show such a large difference between the species. But we have seen that in *Orchesella*, the effect is primarily on growth, and in *Platynothrus* it is primarily on reproduction. The NOEC for population growth of *Platynothrus* clearly is more sensitive than for *Orchesella*.

Table 4 *Comparison of criteria obtained from cadmium toxicity experiments, combined with life-history information to estimate the population growth rate*

Critical level of cadmium in food (µg/g)	*Orchesella cincta*	*Platynothrus peltifer*
LC_{50} (9 weeks)	180	817
NOEC (growth, reproduction)	4.7	2.9
NOEC (population growth)	56	2.9

After Van Straalen *et al.* (1989).

This comparison leads to the clear conclusion that the LC_{50} may give a totally wrong indication of the differences between species in their susceptibility to toxicants. Of course, population growth is the most relevant criterion for the prediction of effects in the field. The LC_{50} is often determined in toxicity experiments, but it does not allow a straightforward extrapolation to the field. In the present example, conclusions on the differences between species are the opposite to what would be expected on the basis of the LC_{50}.

The response of the population growth rate reflects a difference in strategy between the two species. When *Orchesella* is confronted with cadmium in the food, it tries to lay as many eggs as possible, until it dies. Protection of egg production is its top priority. However, by doing this, *Orchesella* is not able to prevent cadmium intoxication, and the LC_{50} is low.

When *Platynothrus* is confronted with cadmium in the food, it first stops producing new eggs, because this costs too much energy. Survival is top priority. By doing this, *Platynothrus* is better able to resist cadmium, but the loss of reproduction has a drastic effect on population growth.

These two strategies are typical for these two species. It would be interesting to see how representative such a trend may be for the soil invertebrate community as a whole. This is a difficult question, but there are some interesting patterns among Collembola especially, that may be relevant.

7. Ecological classifications

Figure 5 shows six species of springtails, which are classified according to three categories; epigeon, hemiedaphon, euedaphon. This classification refers to the average position of the species in the soil profile: the epigeon lives on the soil, the hemiedaphon lives 'halfway' in the soil, and the euedaphon lives deeper in the soil (Christiansen 1964). The upper group has colour patterns, long legs, and a well developed furca, while the lower species are white, they have no eyes and often no furca. The groups are called life-forms.

This classification of the springtails is dated, but in the light of ecotoxicology it may be relevant, because life-history patterns are correlated with life-forms. The surface-active species have higher metabolism, higher fertility and higher population turnover. *Orchesella cincta* clearly belongs to the upper group. The lower group, the true soil inhabitants, have lower fertility, lower metabolism and low population turnover. *Platynothrus*, although it is not a springtail, resembles this group.

If we apply this ecological classification system to ecotoxicology, we may expect that the upper group will be relatively indifferent to persistent toxicants, while the lower group will be more susceptible. Whether this is actually true or not cannot be confirmed on the basis of present evidence. Extrapolation to the field situation has to deal not only with effects at the population level but also with effects due to interactions between species. Vink (1994) has shown that inter-

actions between soil invertebrates and soil microflora may indeed be important for effects of toxicants under more natural conditions.

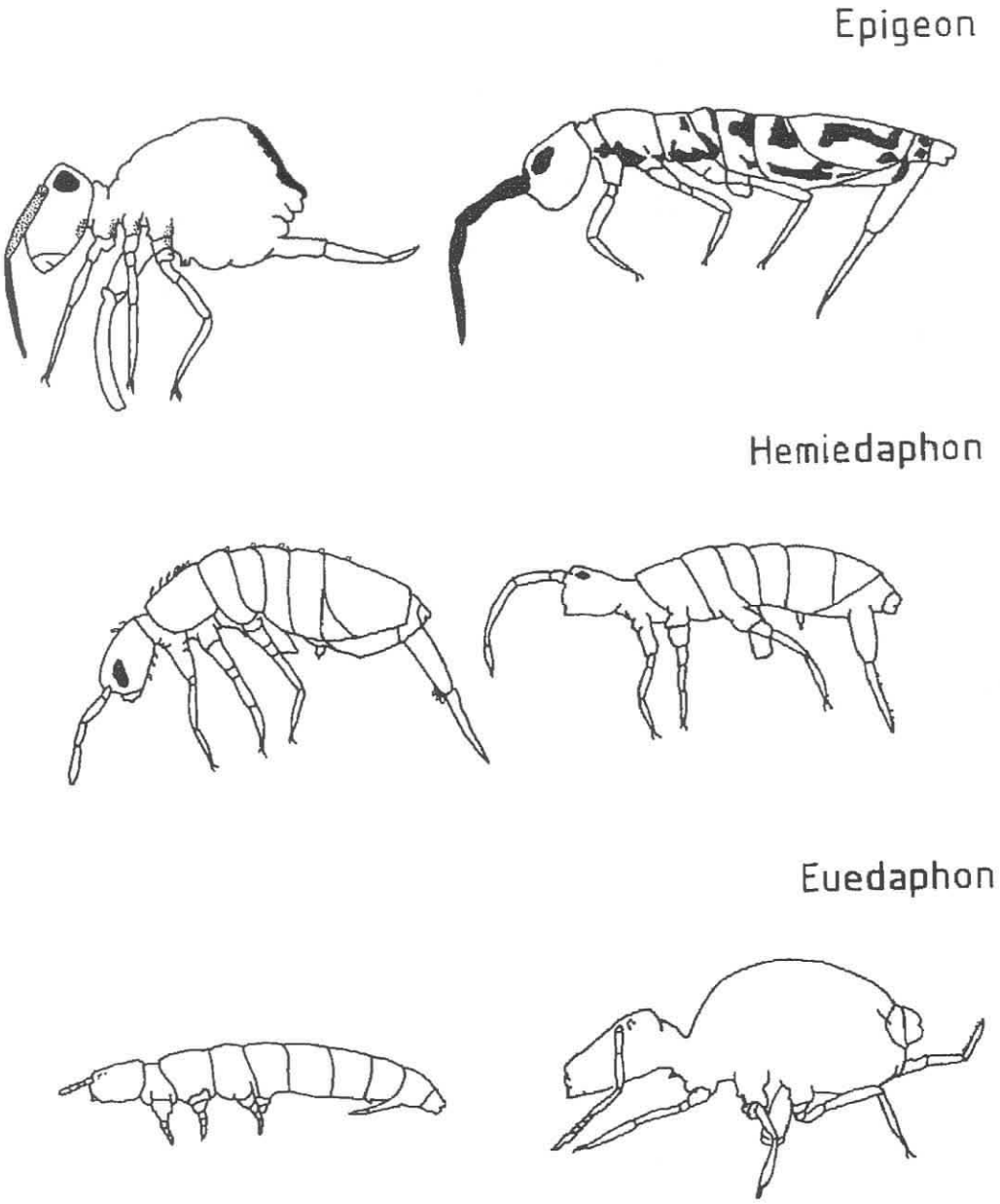

Figure 5 *Some examples of life-forms of Collembola*
After Gisin (1960)

The above arguments lead to three main conclusions. In the first place, life-history information is vitally important for the evaluation of the population consequences of toxic effects. If we use only the LC_{50}, we may arrive at wrong conclusions. Secondly, to obtain bioindicators for soil pollution, the best way to do this will be to classify species according to life-forms, because these seem to be correlated with susceptibility patterns. In the third place, soil contamination will favour opportunistic species with a high reproductive output, even if these are more sensitive in terms of their LC_{50}.

A more extensive comparison of species is needed before these conclusions may be fully validated. There is a great need for more information on toxicity of chemicals in soil, in order to achieve better soil quality criteria (Widianarko *et al.* 1994). Unfortunately, the data base for soil ecotoxicology is not very large, although the community of invertebrates provides an excellent opportunity to study questions of ecotoxicological responses at the population level.

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